Original Article

In vitro Antioxidant and Antibacterial Activities of *Jatropha heynei* Leaf Extract and GC-MS Profiling

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ABSTRACT

Aim: *Jatropha heynei* N. P. Balakr. of family Euphorbiaceae is an endemic herb with ethno medicinal values has not been studied for phytochemical analysis. The present study investigates the antibacterial, antioxidant activity and GC-MS profiling of *J. heynei* leaf extract. **Materials and Methods:** The cyclic voltammetry technique was employed to study the antioxidant activity of leaf extract. *In vitro* antibacterial activity was done by agar well diffusion assay. Further, Chemical profiling of methanolic leaf extract of *J. heynei* was done by the GC-MS analysis. **Results:** Cyclic voltammograms of extract revealed two anodic peaks at 0.12 and 0.22V when anodic current was applied at scan rate of 50 mV s^{-1}. This low oxidation peak potential in the extract suggested its good antioxidant property. *In vitro* antibacterial activity extract showed good inhibitory effect against *Staphylococcus aureus* with zone of inhibition 14±0.57 mm and *Pseudomonas aeruginosa* with zone of inhibition 10±0.25mm. GC-MS analysis of leaf extract revealed presence of 14 prominent chemical compounds. Among them, major abundant compounds includes pentadecanoic acid, phytol, cis, cis, cis-7, 10, 13-hexadecatrienal Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, γ-Sitosterol and Lupeol. Five compounds such as D-chiro-Inositol 3-O-(2-amino-4-((carboxyiminomethyl) amino)-2, 3, 4, 6-tetradeoxy-.alp, phytol, cis, cis, cis-7, 10, 13-hexadecatrienal, vitamin E acetate and β-sitosterol were associated with antioxidant property and compounds like 1, 2, 3-propanetriol monoacetate, octadecanoic acid, phytol, 2,6,10,14,18,22-Tetracosahexaene, and 2,6,10,15,19,23-hexamethyl-, (all-E) were known to posesss antimicrobial activity. Several other compounds with a number of therapeutic properties were also reported in the present study.

Keywords: GC-MS analysis, Cyclic voltammetry, Antibacterial activity, *Jatropha heynei*, Euphorbiaceae.

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INTRODUCTION

Antioxidants are chemicals that neutralise harmful free radicals and other chemically active metabolic by products. Plant phenols and polyphenols, with their antioxidant potential, play an important role in the prevention of several human disorders, including cancer, cardiovascular disease, and neurological disease, which are thought to be caused by oxidative stress.[1] and plant phenols were belived to be effective against cancer, viruses, and bacterial diseases.[2] Since synthetic antioxidant chemicals used in the food industry are unstable and highly volatile, their safety and efficacy are frequently being questioned.[3] As a result, the search for naturally occurring antioxidants capable of protecting humans from oxidative stress-induced damage has intensified.[4] The electrochemical measures provide benefits for determining antioxidant property, such as its usage as a quick indicator of the antioxidant activity of a large number of metabolites. The cyclic

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voltammetry (CV) oxidation potentials have been used to assess the antioxidant strength of substances such as phenolic acids, flavonoids and other class of secondary metabolites.[5]

Infectious diseases caused by microorganisms are a major cause of death and morbidity in humans. Despite the fact that various antibiotics have been created to treat these diseases with maximum efficacy, their mismanagement and misuse, as well as microbial evolution, have resulted in the rise of drug-resistant strains. Hence, antibiotics known to heal specific diseases have lost their potency during the last few decades. As a result, the hunt for new antibacterial medications derived from natural sources is justified.[6]

There are about 175 species within the genus *Jatropha* and it belongs to spurge family Euphorbiaceae. Originating in tropical America, Jatropha species are now found throughout the tropics and subtropical regions of Asia and Africa,[7] where they have been used in traditional medicine to treat a number of illnesses.[8] Some *Jatropha* species, like *Jatropha curcas* and *Jatropha mollissima*, are annuals, but most of them are perennials. The *Jatropha* species have a wide range of habits, including shrubs, trees, and succulents.[9] Several species of this genus have already been studied for their pharmacological activities and metabolite profiling. Pharmacological activities of the *Jatropha* genus have shown links between bioactive molecules and a number of bioactivities, such as antioxidant[10] antimicrobial,[11] antihelmintic,[12] cytotoxic[13] and anagelsic.[14] Chemical studies of *Jatropha* species have resulted in the discovery of monoterpenes, sesquiterpenes, cyclic peptides, lignans, sesquineolignas, flavonoids, coumarins, coumarino lignans, and alkaloids. Even though the genus *Jatropha* is important, only a few species have been tested chemically. *Jatropha heynei* is a rare and endemic herb found in Southern India growing in a nutrient rich well-drained soil. The *J. heynei* has attributed some medicinal values and used by local people of Chitradurga, Karnataka, India. In this context, we have evaluated *in vitro* antibacterial, antioxidant activity and metabolite profiling of *Jatropha heynei* leaf extract by GC-MS analysis.

MATERIALS AND METHODS

Collection of plant material and identification

The fully matured *J. heynei* plant (Figure 1) samples were collected from Surammanahalli village, Chitradurga district of Karnataka, India. The plant was identified by Department of Applied Botany, Kuvempu University and confirmed by referring Gamble flora volume 3. The healthy plant samples were collected in sterile polythene bags and processed to the laboratory within 24 hr. The herbarium specimen with voucher no-KU/AB/05 was deposited to Kuvempu University.

Figure 1: Habit of the *Jatropha heynei*.

Preparation of plant extract

The collected healthy plant samples were first washed in running tap water to remove adherents and air-dried in shade for 20-25 days. Leaf samples were then finely powdered using home grinder and subjected to soxhlet extraction using methanol solvent.[15] The methanolic crude extract was filtered, then evaporated in the open air. The extract was dissolved in DMSO (Dimethyl Sulfoxide) and refrigerated until use.

Antioxidant activity by cyclic voltammetry method

The Electrochemical Workstation CHI 660c determined electrochemical measurements. Utilizing a standard three-electrode system and a single-compartment cell, cyclic voltammetric measurements were conducted. The three-electrode system consisted of carbon paste working electrode (CE), saturated calomel reference electrode, and a platinum wire auxiliary electrode. To investigate the effect of various scan rates on the anodic oxidation of the compounds in extract, potassium chloride (1 M, 2 mg) was applied to the electrode prior to scanning. At a scanning rate of 50 mV s^{-1}, the redox potential behaviour of extracts was evaluated. Plant extract was prepared by dissolving 2 ml of extract in a p^H 7.0 phosphate buffer containing 65% w/v 50 mM disodium hydrogen phosphate and 35% w/v 50 mM sodium dihydrogen phosphate. Signals were recorded voltammetrically at room temperature.[16]

In vitro antibacterial assay

The antibacterial potential of the leaf extract was tested against two clinical bacterial strains obtained from Chandigarh's Institute of Microbial Technology (IMTECH). Gram-positive strain *Staphylococcus aureus* (MTCC 902) and Gram-negative strain *Pseudomonas aeruginosa* (MTCC 4734) were studied using the agar well diffusion method. Standard antibiotic ciprofloxacin was used as positive control and dimethyl sulphoxide (DMSO) was used as the negative control. Leaf extract (10 mg ml^{-1}) was dissolved in DMSO and made concentrations of 100%, 50%, and 25% were prepared. A sterilised cork-borer was used to create wells (0.5 mm) in the solidified nutrient agar media, 20 µl of the extract was poured into each well and then kept for incubation for 24 hr at 37°C. For the purpose of assessing the antibacterial properties, the zone of inhibition (ZI, mm) on plates was determined.[17]

Phytochemical profiling by GC-MS analysis

To determine chemicals present in *J. heynei* leaf extracts, gas chromatography combined with mass spectrometry (GC-MS) was used. For this analysis, we used a gas chromatograph (Agilent Technologies 6890 N Network GC) and mass detector (5973 Network Mass Selective Detector) operating in 70 eV electron impact (EI) mode. The analytes were separated using gas chromatography with a 30-meter, 0.25-millimeter, and 0.25-meter Agilent HP-5 MS capillary column. The temperature programme started at 50 degrees Celsius and increased to -280 degrees Celsius at a rate of 6 degrees Celsius per minute for every minute of holding at each new temperature. 1.0 mL min^{-1} of helium was used as the carrier gas. As soon as the components were isolated, MS detection was initiated. The electron ionization source of 70 eV was generated by MS. Both the MS transfer line and source were heated to temperatures of 280 degrees Celsius. When the MS quadrupole was heated to 150 degrees, the temperature rose to 450 degrees. As an electron ionisation source, the MS was able to (70 eV). The full-scan acquisition mode was used for the qualitative analysis. The identification of intermediate products was made possible by comparing the mass spectra to those found in the NIST 98 Library.

RESULTS

Antioxidant activity by cyclic voltammetry

Cyclic voltammetry (CV) is probably the most common voltammetric technique for analysing redox systems. Cyclic voltammetry was used to test the reducing capability of extracts where there was a strong association of redox potential with antioxidant activity. Figure 2 depicts the cyclic voltammogram of leaf extract. Inverting the scan direction revealed two anodic peaks for the extract and one cathodic peak, suggesting the reversibility of the oxidation reaction of the extracts. The anodic peak was generated between 0.12 and 0.22 V at pH 7 and 50 mV/s scan rate. The shift of anodic peak potential towards a positive value indicates the existence of oxygen radical scavengers in the extract.[18]

The measurement was carried out at a P^H of 7.0 using a carbon paste electrode with a diameter of 3 millimetres as the working electrode, saturated calomel as the reference electrode, and a platinum wire as the auxiliary electrode. The scan rate was set at 50 milli volts per second.

In vitro antibacterial activity

The antibacterial effect of crude extract against clinical bacterial strains was done by agar well diffusion assay. The extract showed considerable antibacterial activity against Gram positive bacteria *Staphylococcus aureus* with zone of inhibition of 14±0.57 mm and Gram-negative bacteria *Pseudomonas aeruginosa* with zone of inhibition of 10±0.25 mm at the higher concentration of extract (Figure 3).

Metabolite finger printing by GC-MS analysis

GC-MS chromatograms (Figure 4 and Table 1) revealed the presence of 30 compounds with various biological activity in the plant extract. The major constituents present in the methanolic leaf extract are- D-chiro-inositol 3-O-(2-amino-4-((carboxyiminomethyl) amino)-2, 3, 4, 6-tetradeoxy-.alp, Phytol, cis, cis, cis-7, 10, 13-hexadecatrienal, vitamin E acetate, β-sitosterol, 1, 2, 3-propanetriol, monoacetate,

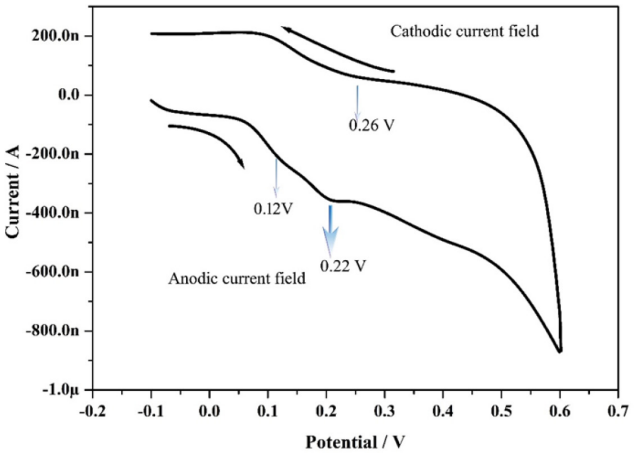

Figure 2: Voltammogram of the methanolic leaf extract of *J. heynei*.

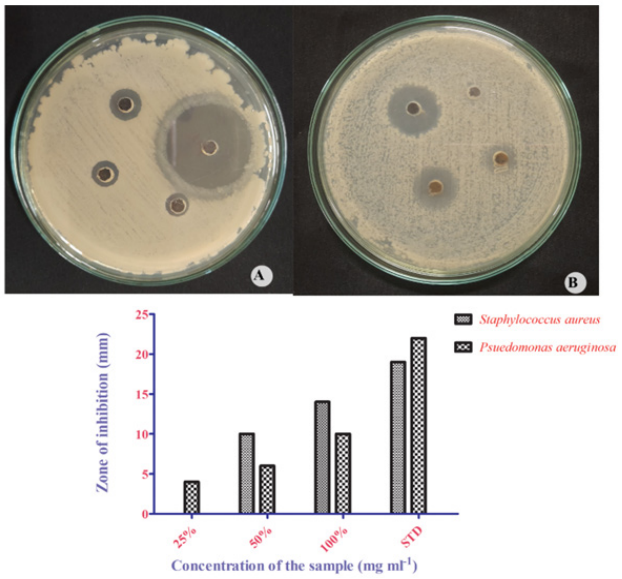

Figure 3: Antibacterial activity of *J. heynei* leaf extract against (A) *Staphylococcus aureus* (B) *Psuedomonas aeruginosa*.

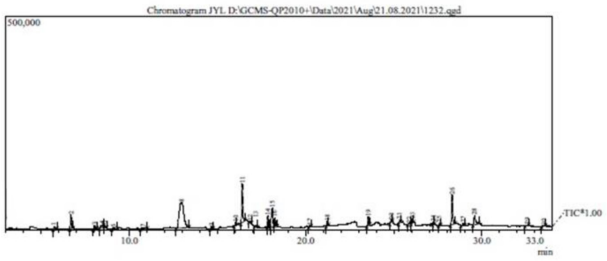

Figure 4: Chromatogram of GC-MS analysis of *J. heynei* leaf extract.

Octadecanoic acid, 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E), Pentadecanoic acid, Lupeol, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 1-Butanol, 4-butoxy, Pentanoic acid, 3-[(adamantan-1-ylmethyl) carbamoyl]-4-phenyl and gamma-Sitosterol.

DISCUSSION

The cyclic voltammetry (CV) technique was used to measure the antioxidant capacity of methanolic leaf extracts. One major drawback of spectrophotometric methods is that they can be affected by interference from bio-molecules that absorb at the same wavelength as the test being performed, which can skew the results[19] However, the electrochemical test that allows the detection of the substantial oxidation potential of particular substances at different pH levels and in various reaction conditions shows its uniqueness in assessing the antioxidant activity. In addition, the cyclic-voltammetric method may serve as an alternative to spectrophotometric techniques.[17] In the present study two peaks at 0.12 and 0.22 V indicated the high oxidation potential of extract in the presence of anodic current suggested its significant antioxidant capacity. Compounds with two or more electron-donating groups have a low anodic peak potential and high antioxidant abilities. In this study, compounds with a low anodic peak potential could have strong antioxidant properties. Antioxidant compounds are known to play a new role in treating cell damage caused by reactive oxygen species.[36] The similar observations were supported by previous research work.[37] The presence of flavonoids and phenolic components in an extract is indicative of its high antioxidant activity.[20]

The well diffusion method is more appropriate for determination of antibacterial activity, compared to the method of disc diffusion. The leaf extract showed moderate antibacterial effect to both the bacterial strains *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The extract showed more activity to Gram positive bacteria *Staphylococcus aureus* when compare to *Pseudomonas aeruginosa*. The resistance of Gram negative bacteria could be attributed to their cell wall structure. Gram-negative bacteria have an effective permeability barrier composed of a thin lipopolysaccharide exterior membrane, which could limit the penetration of the plant extract. It has previously been reported that Gram-negative bacteria are typically more resistant to plant-origin antimicrobials and even show no effect when compared to Gram-positive bacteria.[38] Colony inhibition in the bacterial strains might be brought about by suppression of DNA or protein synthesis or damage to the integrity of cell membranes, which ultimately leads to cell death.[21] A relationship between antibacterial activity and chemical composition was previously demonstrated [39] and findings of present work supported by previous research work suggested that the inhibitory effect of extract against pathogenic bacterial strains could be associated with the presence of active antimicrobial compounds and synergistic effect of other bio-active compounds present in the extracts.[40] Observations of the antibacterial and antioxidant activites in the leaf extract motivated the authors to investigate crude extracts for chemical profiling using GC-MS analysis..

Medicinal plants are a source of novel treatments, and many modern medications are derived indirectly from medicinal herbs. Numerous contributions have been made fight against various diseases and disorders. *J. heynei* is rare herb and has not been studied for the phytochemical analysis. Hence the present study

Table 1: Metabolite profiling of *J. heynei* leaf extract by GC-MS analysis.

RT	Compound name	Molecular weight	Molecular Formula	Biological activities
5.76	Cyclopentane, 1-acetyl-1,2-epoxy-	126	$C_7H_{10}O_2$	No reports
6.70	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	$C_6H_8O_4$	No reports
8.04	1,2,3-Propanetriol, monoacetate	134	$C_5H_{10}O_4$	Antifungal[27]
8.46	Propanoic acid, 3-(acetylthio)-2-methyl-	162	$C_6H_{10}O_3$	No reports
8.61	Nitro-tert-butyl-acetate	161	$C_6H_{11}NO_4$	No reports
9.09	Heptanol	116	$C_7H_{16}O$	No reports
10.80	1-Butanol, 4-butoxy-	146	$C_8H_{18}O_2$	Volatile Biomarker for gastric cancer[33]
12.97	Dodecanoic acid, 3-hydroxy- beta-Hydroxydodecanoic acid	216	$C_{12}H_{24}O_3$	No reports
14.66	3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	280	$C_{14}H_{16}O_2$	No reports
16.04	Heneicosanoic acid, methyl ester	340	$C_{22}H_{44}O_2$	No reports
16.41	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	Anti-inflammatory[30]
16.84	D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl)amino)-2,3,4,6-tetradeoxy-.alp	379	$C_{14}H_{25}N_3O_9$	Antioxidant[22]
17.16	Beta.-D-Mannofuranoside, 1-O-(10-undecenyl)-	332	$C_{17}H_{32}O_6$	No reports
17.86	Phytol	296	$C_{20}H_{40}O$	Antioxidant, anti-inflmmatory, antimicrobial[23]
18.12	Cis,cis,cis-7,10,13-Hexadecatrienal	234	$C_{16}H_{26}O$	Antioxidant[24]
18.28	Octadecanoic acid	284	$C_{18}H_{36}O_2$	Antibacterial[28]
20.24	Trichloroacetic acid, tridec-2-ynyl ester	340	$C_{15}H_{23}Cl_3O_2$	No reports
21.22	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330	$C_{19}H_{38}O_4$	Anti-inflammatory[32]
23.56	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	410	$C_{30}H_50$	Antibacterial[29]
24.87	Silicic acid, diethyl bis(trimethylsilyl) ester	296	$C_{10}H_{28}O_4$	No reports
25.33	N-Methyl-1-adamantaneacetamide 2-(1-Adamantyl)-N-methylacetamide	604	$C_{34}H_{49}ClO_7$	No reports
25.86	2-[4-Cyclohexylbutanoylamino]-3-chloro-1,4-naphthoquinone	359	$C_{20}H_{22}ClNO_3$	No reports
26.04	Vitamin E acetate	472	$C_{31}H_{52}O_3$	Antioxidant[25]
27.25	Beta.-Sitosterol	414	$C_{29}H_{50}O$	Anti-inflammatory, Anti-diabetic, Anti-oxidant,[26]
27.59	Gorgost-5-en-3-ol, (3.beta.)-	426	$C_{30}H_{50}O$	No reports
28.31	Gamma.-sitosterol	414	$C_{29}H_{50}O$	Antidiabetic[35]
28.94	5H-3, 5a-epoxynaphth [2, 1-c] oxepin, dodecahydro-3, 8, 8,11a-tetramethyl-, [3S-(3.alpha.,	278	$C_{18}H_{30}O_2$	No reports
29.58	Lupeol	426	$C_{30}H_{50}O$	Anticancer, antiprotozoal, anti-inflammatory[31]
32.56	Benzene, 2-[(tert-butyldimethylsilyl)oxy]-1-isopropyl-4-methyl-	264	$C_{16}H_{28}O$	No reports
33.49	Pentanoic acid, 3-[(adamantan-1-ylmethyl)carbamoyl]-4-phenyl-	369	$C_{23}H_{31}NO_3$	Obesity[34]

was undertaken to find out the bioactive compounds present in the methanolic extract of *J. heynei* by using Gas chromatography and Mass spectroscopy. GC-MS analysis of crude extract showed the presence of various bio-active compounds. Among them compounds such as D-chiro-inositol 3-O-(2-amino-4-((carboxyiminomethyl) amino)-2, 3, 4, 6-tetradeoxy-.alp, phytol, cis, cis, cis-7, 10, 13-hexadecatrienal, vitamin E acetate and β-sitosterol were associated with antioxidant property.[22-26] Further, several other compounds with various biological properties were also reported. Compounds like 1, 2, 3-propanetriol, monoacetate, octadecanoic acid, phytol and 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- were known to posesss antimicrobial activity.[27-29] Some compounds like pentadecanoic acid, phytol, β -sitosterol, and lupeol were reported to possess anti-inflammatory activity.[26,30,31] Among the above

compounds phytol, β-sitosterol, and lupeol were associated with multiple biological properties. In addition, certain compounds like - hexadecanoic acid and 2-hydroxy-1-(hydroxymethyl) ethyl ester have medicinal roles such as arachidonic acid inhibitor and increase aromatic amino acid decarboxylase activities. Increase in aromatic amino acid decarboxylation will improve the production of Dopa which is required for better neurotransmission and pain relief. Inhibition of arachidonic acid leads to reduction in production of proinflammatory cytokines, which help in reduction of allergic reactions and inflammation.[32] Compound 1-butanol, 4-butoxy- was used as volatile biomarker for gastric cancer[33] and pentanoic acid, 3-[(adamantan-1-ylmethyl) carbamoyl]-4-phenyl- was used for obesity.[34] Another compound gamma-sitosterol is has antidiabetic property.[35] The present study revealed the presence of several compounds with antioxidant, antimicrobial and other biological activities in the extract of *J. heyeni*.

Many modern drugs are indirectly derived from medicinal plants helped to fight against many human diseases. *J. heynei,* medicinal herb has not been studied. The present study used Gas chromatography and Mass spectrometry to identify a number of compounds with bioactive principles in methanolic extract that could be used in formulation of drugs. Further, the extract showed promising antioxidant and antibacterial properties which upholds the importance of this plant species.

CONCLUSION

In the present study GC-MS analysis of methanolic extract of *Jatropha heynei* showed presence of 14 active metabolites with different biological activites in the extract and supports the ethno-medicinal applications of the plant species for treating various diseases and conditions. The extract also showed significant oxidantion potential by cyclic voltametric method which indicated good antioxidant source. Further, extract also exhibited antibacterial property against tested bacterial strains. Thus *J. heynei* could be a significant source of important compounds potential source of therapeutic agents which can be used in formulation of drugs by the pharmaceutical industries.

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CONFLICT OF INTEREST

There are no conflicts of interest in this research work.

ABBREVIATIONS

GC-MS: Gas Chromatography Mass Spectroscopy; **CV:** Cyclic Voltammetry; **NIST:** National Institute of Standards and Technology; **DMSO:** Dimethyl Sulphoxide; **MTCC:** Microbial Type Culture Collection; **MS:** Mass Spectroscopy.

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